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MICROBIOLOGICAL INDICATORS IN THE WATER AND SEDIMENT OF THE SHALLOW PANONNIAN LAKE - LAKE PALIC

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Abstract

The Palic Lake is a shallow Pannonian lake, whose morphology and natural recharge have been changed decades ago. Reclamation work across the drainage basin of the Lake influenced loss of the most important natural water recharge. In the Lake's surroundings watercourses were regulated and open canal network was constructed, consequently the floodplains disappeared and just several wet meadows remained. Today, the Lake has recreational purposes but it is also a collector for treated municipal waste waters. The consequences of the discharge of treated and untreated wastewater is polluted water with high trophicity and large amount of sediment. The most important fact is that the Lake is a part of a natural park and a habitat for waterbirds (IBAs site). Numerous relict and endemic species, as well as all living organisms are affected by eutrophication and poor water quality. Contents of faecal coliforms and enterococci were determined in order to detect contamination sources. Methods for determination of microbiological quality of water and sediment: detection of faecal coliforms and enterococci has been done with the Colilert-18 and Enterolert-E test, respectively. The results show that the number of bacteria decreases downstream (the lake discharges in manmade canal), and indicate potential "hot spots" for swimmers. The content of faecal coliforms in the water samples goes from the 41-435.200 MPN/100 ml and in the sediment 9-66 MPN/g DW; the content of enterococci goes from 10-15.960 MPN/100 ml and in the sediment 18-50 MPN/g DW.

Keywords: water and sediment quality, fecal coliforms, enterococci, shallow lake, nature reserve

1 INTRODUCTION

"Wetlands are still confined to the 'nature conservation' box" is stated in the report of the World Wide Fund For Nature (2003). The same source confirms that the most of the European countries considered the integration of wetlands into water management as inadequate, which leads to uncoordinated actions in managing wetlands and missed opportunities for fully exploiting their positive role in water management. Wetlands should be included in water management strategies and plans, since the aquatic ecosystems are often affected by human actions, considering both quality and quantity. Examination site presented in this paper is not just affected by human activities, but entirely changed, which will be described in following chapters.

Recreational waters and beaches contaminated with faecal bacteria may contain pathogens that pose a health risk to humans (Hansen et al., 2009, United States Environmental Protection Agency, 2012). Many members of the total coliform group and some so-called faecal coliforms are not specific to faeces and even *E. coli* has been shown to grow in some natural aquatic environments (Ashbolt et al., 2001). Ksoll et al. (2007) listed various literature that proves that faecal coliforms and *Escherichia coli* can persist in secondary, non-host habitats. Therefore the primary targets representing faecal contamination in temperate water are now considered to be *E. coli* and enterococci (Ashbolt et al., 2001; United States Environmental Protection Agency, 2012).

Prolonged survival of faecal coliforms and *E. coli* in freshwater, as well as survival of *E. coli* in sediments and soils over extended periods of time has already been indicated in several studies (Ksoll et al., 2007). The abundances of faecal coliforms and *E. coli* in periphyton communities were positively correlated with water temperature and furthermore, temperature shifts in natural environments may induce a cold shock response and may be one reason why faecal coliforms and *E. coli* persisted through the winter in the periphyton communities (Ksoll et al., 2007).

It is usually difficult to determine the sources, pathways or spread, before the intake of microorganisms by individuals or populations, mainly due to their nature. The dynamic nature of microorganisms is a characteristic that differentiates microbial exposure assessment from chemical exposure assessment (US EPA & USDA, 2012), since changes in the number of microorganisms are influenced by different factors (environmental conditions, food web, etc.). The aim of this research is to indicate contamination sources and potentially “hot spots” for swimmers.

2 METHODS
2.1 Site description

The Palic Lake is a shallow Pannonian lake, whose morphology and natural recharge have been changed decades ago. Reclamation work across the drainage basin of the Lake influenced loss of the most important natural water recharge. In the Lake’s surroundings watercourses were regulated and open canal network was constructed, consequently the floodplains disappeared and just several wet meadows remained. Today, the Lake has recreational purposes but it is also a collector for treated municipal waste waters. The consequences of the discharge of treated and untreated wastewater is polluted water with high trophicity and large amount of sediment. Numerous diffuse and point sources of nutrients caused accelerated eutrophication, which further resulted in formation of thick layers of nutrient-enriched sediment (Raicevic et al., 2011). The Palic Lake is a eutrophic lake, with high self-cleaning ability, but it is being loaded with excessive amount of nutrients, therefore the nutrient concentrations in water are always high (Raicevic et al., 2012).

The site is a popular recreation area for swimming, sailing and rowing, although recently the signs with warnings that water is not for swimming are frequently posted at the coast during the summer, due to high levels of faecal coliforms. The Lake is frequently visited by numerous species of waterbirds, therefore the Lake became a part of a natural park and a habitat for waterbirds (IBAs site). Data regarding fauna indicate that the mixture of human induced and changed natural habitats of the protected area represent the last refuge to endangered species whose habitats are ruined by regulation of watercourses and by spreading of forest monoculture (Pokrajinski zavod za zaštitu prirode, 2011).

2.2 Sampling and analyses

Sampling was done during November (2013), after the summer season, on several locations. The selected locations are along the coastline, since these are the places where the population may be in contact with lake water and sediment, and potentially exposed to waterborne diseases. Moreover, wastewater is usually discharged in the vicinity of these areas. Sampling locations are displayed on Figure 1. Sediment sampling was done just on two locations (L3 and L4). There was no deposited sediment at the point of effluent discharge and sampling point L2 was in that manner unreachable.

As stated before, *E. coli* and *Enterococcus* are the most widely used indicator organisms to determine water quality. The content of coliforms and *E. coli* are usually correlated, therefore the contents in water and sediment were determined. Methods for determination of microbiological quality of water and sediment: detection of total and faecal coliforms and *E. coli* has been done with the Colilert-18 test and detection of enterococci has been done with the Enterolert-E test, both developed by IDEXX Laboratories, Inc. Both methods are based on nutrient indicators and their utilization by tested organisms. The samples were kept under 8^0C in the refrigerator during transit to the laboratory. Prior to testing water samples were diluted to 10^{-2} and 10^{-3}. In order to prepare sediment samples, 30g of sediment was poured into 270 mL of sterile water, and then put on rotation mixer to shake for 45 minutes. After shaking, samples were diluted to 10^{-2} and 10^{-3}. The diluted samples were tested afterwards by standard procedures of Idexx Colilert and Enterolert.

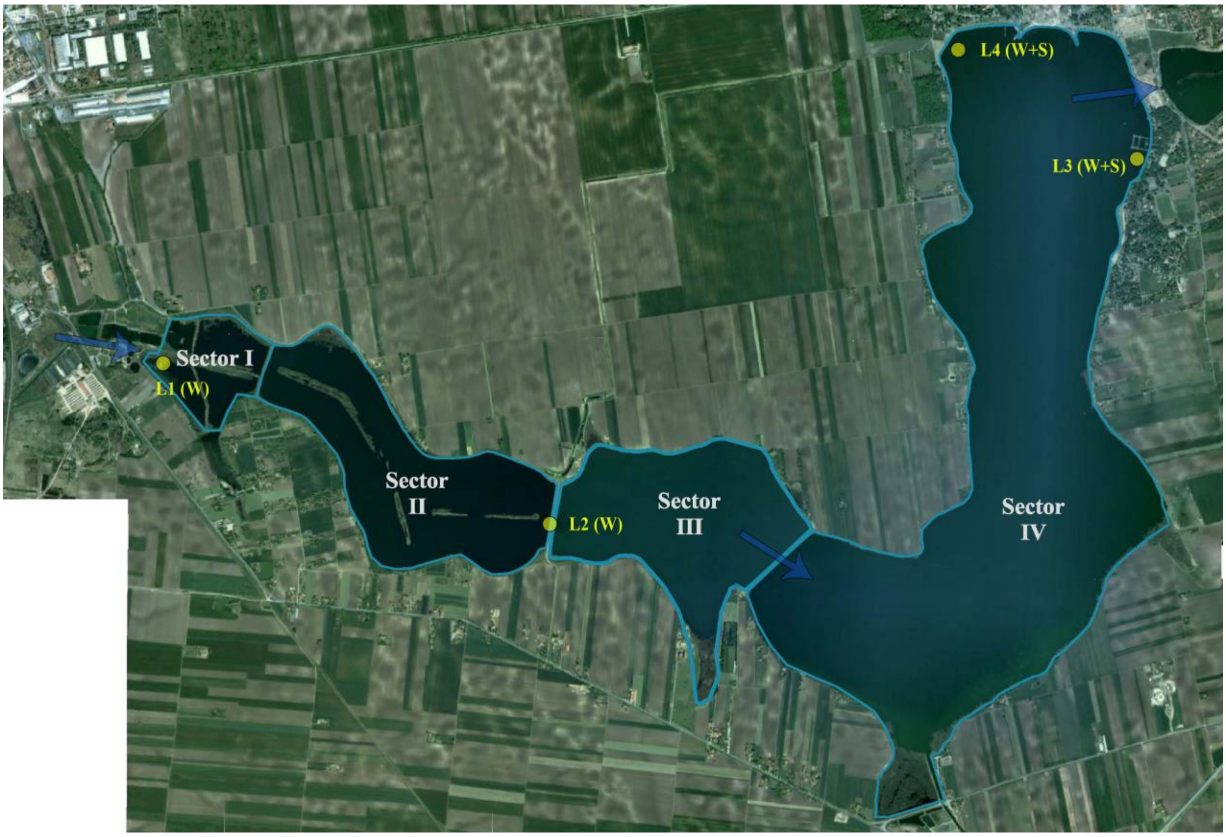

Figure 1. Sampling sites and water flow direction (W-water samples, S-sediment samples)

3 RESULTS AND DISCUSSION

The samples were taken in November, although the weather conditions, particularly temperatures were extraordinarily high for this time of year (Table 1). Basic water quality parameters, measured in-situ are also displayed in Table 1. The pH value goes from neutral (effluent-L1) to alkaline (lake water L2-L4). Lake water has higher values of electrical conductivity at locations L3 and L4. Dissolved oxygen readings that show super-saturation and high water turbidity are evidence of high algal content, even in this part of the year.

Table 1. In-situ measurements

	Unit	L1	L2	L3	L4
T_{air}	°C	11	11	11	13
T_{water}	°C	16.5	13.5	13.1	13
EC_w	μS/cm	819	875	1056	1057
pH		7.47	8.97	8.99	8.93
O_2	mg/L	4.5	11.04	11.06	8.73
Air saturation	%	47	107.7	106.9	84.3

Contents of faecal coliforms and enterococci were determined in order to detect contamination sources. The results show that the number of bacteria generally decreases downstream (Figure 2), since the lake discharges in manmade canal and there is always a low gradient that promotes the flow. Bacterial content generally reduces along the flow of the water, except at the last sampling site, L4, where the content of all examined parameters in water raises. The bacterial content in effluent is particularly high. The content of faecal coliforms in the water goes from the 41-435.200 MPN/100 mL and in the sediment 9-66 MPN/g DW; the content of enterococci goes from 10-15.960 MPN/100 mL and in the sediment 18-50 MPN/g DW (Table 2).

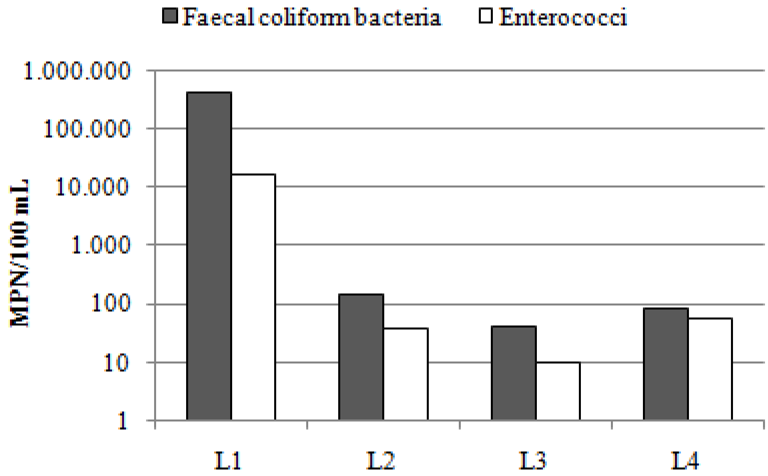

Figure 2. Spatial distribution of faecal coliforms and enterococci in the lake

Limit values according to the Serbian Regulations on limits of pollutants in surface and ground water and sediment and deadlines for their accomplishment for lakes of good ecological status (second class), are as follows, for total coliforms 10000 CFU/100 mL, faecal coliforms 1000 CFU/100 mL and enterococci 400 CFU/100 mL. Content of listed bacteria in water of the Palic Lake is consistent with mentioned limits. When the samples are evaluated as water for recreational purposes, sharper limits should be used, as US EPA (2012) standards that assume up to 126 *E. coli* per 100 mL and 33 enterococci per 100 mL. The content of *E. coli* in water is acceptable, although the content of enterococci is somewhat higher. Concerning the fact that the samples were not collected during the summer season, these facts could be disregarded. For the months from November through April, the limits for recreational purposes may be disregarded. On the other hand, it should be kept in mind that enterococci provide valuable data on the bacteriological quality of water, especially because these bacteria rarely multiply in polluted water and are more persistent than *E. coli* (Potgieter, 2007; Standard Methods, 1995).

Table 2. Microbial indicators of water and sediment quality

Parameter	Unit	Efluent	Palic Lake - WATER		
		L1	**L2**	**L3**	**L4**
Total coliform bacteria	MPN/100 mL	770100	461.1	167	648.8
Faecal coliform bacteria	MPN/100 mL	435200	146	41	85
Escherichia coli	MPN/100 mL	160700	9.7	6.3	18.1
Enterococci	MPN/100 mL	15960	36.4	9.5	58.1
			Palic Lake - SEDIMENT		
		L1	**L2**	**L3**	**L4**
Total coliform bacteria	MPN/g DW	-	-	226.3	170.73
Faecal coliform bacteria	MPN/g DW	-	-	65.7	8.89
Escherichia coli	MPN/g DW	-	-	<1.4	4.44
Enterococci	MPN/g DW	-	-	18.1	49.6

Persistence of enterococci in sediment has already been recorded and its abundance is site specific, i.e. it depends on characteristics such as mineralogy, particle size, moisture content, organic matter and nutrient availability (Piggot et al., 2012). The texture of the sediment is sandy, with significant portion of fine sand particles. Sediment samples taken on location L3 have higher content of bacteria in comparison to water samples, except in case of *E. coli*. Sediment materials probably provide bacteria some protection against light-induced inactivation (Hansen et al., 2008). Enterococci attached to upper layer sediments are easily resuspended in the water column, therefore during rough wave conditions, sediment and water enterococcal isolates show greater similarity than during calm conditions (Townsend et al., 2006).

Considering everything mentioned, there is a general conclusion that in the vicinity of L4 exists another source of pollution. Bacterial content in water is higher in comparison to sampling point L3, except in regard to faecal coliforms. Moreover, bacterial content is to some extent higher in water than in sediment.

4 CONCLUSIONS

Results of conducted analyses may be regarded as zero state, or a basis for further research. Conclusions on selected spots may serve as a base for establishing a conceptual model of the Lake for modelling purposes. It is possible to indicate the vicinity of probable source of pollution according microbial indicators in water and sediment, although for higher precision and accuracy, additional monitoring and analyses are needed. It is necessary to determine if the levels of organic material in the sediment correlate with the levels of enterococci in sediment. For this purpose longer data series are needed, for different weather conditions. Coliform can originate from many sources (waterfowl, watercraft, swimmers and bathers), many of which may be difficult to evaluate, especially in urban areas (industrial waste, storm sewer discharge, etc.) (Canale et al., 1973). Monitoring faecal indicator bacteria in recreational waters and developing reliable methods to identify their possible sources require great effort and expense (Hansen et al., 2009). Therefore, future research can be extended to determination of pollution origin by DNA extraction and determination of fingerprints of enterococci in water column and sediment.

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